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A STUDY OF COMPLEMENT FIXATION IN TUBERCULOSIS.

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## A STUDY OF COMPLEMENT FIXATION IN TUBERCULOSIS.

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The problem of the diagnosis of Tuberculosis is a fascinating one, none the less so because there is no well defined division between the tuberculous infection harboured by practically every healthy inhabitant of a civilised country, and that which we are justified in stigmatising as "disease."

In looking for a test to supplement clinical findings, then, we must first of all determine a point at which to aim, and the magnitude and difficulties of the task become at once apparent. The question cannot be "Has the patient tuberculosis" for we can be almost certain that the answer to that is in the affirmative: it must be "Has he tuberculosis that matters?" and it is obvious that from its nature, it is incapable of such a satisfactory reply as the simple "Yes" or "No" of the Wassermann.

Recognising these facts, then, we cannot hope to relieve the clinician of his responsibilities and those optimists who set out to do so seem doomed to failure from the beginning but if we can produce a test which will help his diagnosis in a difficult case, and confirm it in a more obvious one, we shall have achieved a useful end.



Tuberculin naturally suggests itself, but in all its present forms it has already been thoroughly tried out as a diagnostic agent with no very conclusive results, its main fault being its sensitivity to quiescent lesions of no significance. The case of the young child is of course an exception, for here the question "Yes" or "No" being of paramount importance, the test is of great value.

The Complement Fixation Reaction has also had an extensive trial, and perusal of its literature leaves one very much confused as to its value or otherwise. Some investigators obtain startlingly satisfactory results, while others, using the same technique, produce no results at all, though very often the latter will introduce some modifications with which they alone are perfectly satisfied.

Facilities being within my reach at the Royal Victoria Dispensary for Tuberculosis I determined to investigate the possibilities of the procedure, at the same time bearing in mind the limited objective already referred to.



THE PRESENT POSITION.

A Brief review of work already done is necessary for an introduction.

In 1906 Wassermann and Bruck<sup>1</sup> carried out the reaction with the blood of tuberculous individuals using Koch's Old Tuberculin as an antigen. No positive results were obtained unless the patient had previously had tuberculin injections, and meeting with other difficulties they turned their attention to the Complement fixation test in Syphilis.

Since then various investigators have been working along similar lines. Lack of uniformity in results appears to depend on several factors, chief among which are - (a) differences in choice of antigens: (b) variations in technique and method of reading results: (c) differences in material used in testing: (d) varying accuracy in clinical diagnosis: (e) the competence of the person who is carrying out the test. These factors may be appropriately considered at this point in some slight detail.

(A) Differences in Choice of Antigens.

Antigens may be divided into four main groups - (i) Tuberculins: (ii) Emulsions of Living or Dead Tubercle Bacilli: (iii) Extracts of Tubercle Bacilli: (iv) Tuberculous Tissue Extracta. (The last may/



may be dismissed at once, having failed to achieve any success).

(i) Tuberculin antigens were very popular at first. Wassermann<sup>1</sup> himself used such, and later Citron<sup>2</sup> in 1907 and Ludke in 1908, the two latter obtaining inconclusive findings. Eugel and Bauer obtained positives only in tuberculin treated subjects, the reaction becoming negative after the cessation of treatment. The most widely used antigen of this class, however, was Besredka's,<sup>3</sup> this being a filtrate of a culture of tubercle bacilli grown in a glycerine free broth medium with added yolk and white of egg. Inman<sup>4</sup> using this found that positives were generally given by clinically clear patients having a positive Wassermann Reaction, and Bronfenbrenner<sup>5</sup> found the same. Ichok, Goldenberg and Fried<sup>6</sup> used this antigen with success in lupus patients, but did not inquire into its specificity: Debains and Jupille<sup>7</sup> found 90.3 %+ in their "Stage I and II" cases and Meyer<sup>8</sup> also reported upon it.

(ii) Emulsions of Living or Dead Bacilli have perhaps had the most extensive trial. Among the earliest records are those of Bordet and Gengou.<sup>9</sup> Later D'Este Emery, McIntosh and Fildes<sup>10</sup> who published satisfactory results, and Punch<sup>11, 12, 13, 14</sup> and his collaborator, who appear to be very consistent, used/



used simple emulsions prepared by grinding up living bacilli. Besredka's<sup>15</sup> new antigen comes into this class, being an emulsion of dead bacilli, and it was used also by Rieul and Bass<sup>16</sup> whose findings were satisfactory apart from Syphilis and Malaria, by Courcoux<sup>17</sup> examining "other tuberculosis" and also Bass,<sup>19</sup> Fried,<sup>20</sup> Mozer and Fried,<sup>18</sup> Ichok<sup>21</sup> and Kempner.<sup>22</sup>

Dudgeon, Meek and Weir<sup>23</sup> also used a dead emulsion, later however, abandoning this for an extract.<sup>24</sup> A variation of this class is Miller's<sup>25</sup> antigen, which consists of bacilli emulsified with salt, and was used by Watkins and Boynton,<sup>26</sup> Moon,<sup>27</sup> Burns Slack, Castleman and Bailey,<sup>28</sup> Fidler,<sup>29</sup> and Corper and Sweany.<sup>30</sup> Miller himself obtained results apparently almost too perfect to be quite accurate, the others, particularly the last mentioned not being so successful.

It will be noticed that living and dead bacilli have been used with equal success.

(iii) Extracts of Tubercle Bacilli have been favoured by many, but such a number of different methods of preparation have been employed that it is almost impossible to come to any conclusion on the subject.

Craig<sup>31</sup> used a methyl alcohol extract, Petroff<sup>32</sup> using a glycerine extract and also one prepared/



prepared from methyl alcohol. Negre and Bouquet<sup>33, 34</sup> used the latter and preferred it to all others, claiming that it was as sensitive as the ethylic one, easier to prepare, and kept indefinitely. Petroff himself condemned it as being too sensitive. Dudgeon<sup>24</sup> extracted with water and later with alcohol, Mourasound<sup>35</sup> used Petroff's methyl alcohol antigen, Calmette and Massol<sup>36</sup> used a water and peptone preparation, and Corper and Sweany<sup>30, 37</sup> made an antigen of their own by a process of auto digestion with results which, though apparently satisfactory to themselves, seem remarkably poor to a peruser of the literature.

Wang and Crocket<sup>38</sup> also made their own extract with satisfactory findings. This was used by Sellars and Ramsbottom<sup>39, 40</sup> who believed it to be superior to Miller's, Craig's and Dudgeon's. Lastly, Caulfield made use of an alcohol ether extract of the bacilli for the test in conjunction with his inhibitive reaction, for such an antigen is markedly anti-complementary.

#### (L) Variations in Technique and Method of Reading.

These also account for inconsistencies. Some inactivate for 30 minutes at 55°, others (Moon)<sup>27</sup> for only 10 minutes for fear of destroying antibodies: some give 18 hours in a refrigerator and 10 minutes at 38° C. for primary incubation (Ogawa)<sup>41</sup>, others leave/



leave the sera at 38° C for one hour (Punch,<sup>11</sup> McIntosh<sup>10</sup>) or keep the tubes for varying intervals at room temperature, subsequently giving an hour at 38° C (Inman).<sup>12</sup> Some read after one hour's secondary incubation, others after 24 hours in an ice chest.

(C) Differences in Material Used for Testing.

Here age is an important factor, and other things being equal the cases below seven or eight years of age will give fewer positives than those which are older (Cook,<sup>13</sup> Bauer, Heimann<sup>14</sup>) and a series with many children may therefore be expected to be less satisfactory.

In the same way clinically definite advanced cases may give a negative owing to the excess of antigen already in the body (Meyers,<sup>8</sup> Grumbach,<sup>15</sup> Burns, Slack, Etc<sup>16</sup> and Fried<sup>17</sup>), and if this fact is overlooked, and it seems to be so by many, a poor "clinically certain" series will result.

(d) Varying Accuracy in Clinical Diagnosis.

The efficacy of the test is certainly judged by its results in doubtful cases, and the verdict which will be pronounced will therefore largely depend on the observer's own skill in diagnosis.

(e)



(e). The Personal Factor in the Observer.

Lastly, and perhaps most important of all, results depend on the aim and on the competence of the person who is carrying out the work. Craig,<sup>31, 46</sup> Miller,<sup>25</sup> and one or two others obviously intend to have good results, and do so. Corper and Sweany<sup>30, 37</sup> obtaining indifferent success with their own antigen do not try very hard to improve on them with Miller's and Mouround<sup>35</sup> provides what may be regarded as the comic relief of the literature by failing completely to get any result at all, and thus proves to his own satisfaction that results are unobtainable.

Some of the data obtained by workers in more recent times may now be summarised shortly so as to afford a basis for comparison with those set out below. More detailed references when necessary will be given later in connection with the various sections of the investigation.

## (Table 1.)

The following table gives an idea of the average conclusions with regard to pulmonary and other tuberculosis. It is more or less incomplete owing to the fact that the various authors use different classifications, and comparison in tabular form is often impossible.

Table 2 showing groupings according to the duration and activity of the pulmonary lesion, should be more comprehensive.







Table 2.

*Figures indicate % of positive results*

	INCUBIENT.		MOD. ADVANCED		FAR ADVANCED	
	Act- ive.	Quies cent.	Act- ive.	Quies cent.	Act- ive.	Quies cent.
CRAIG	96.7	62.5	98.3	68.7	96.4	100
MILLER			97.2		95.6	
CRAIG	96.2 <i>(all classes active)</i>	58%	-	68	-	100
MOON	87.5		85.7		84.3	
VON WEDEL	100					
STOLL & NEUMAN	37		65		67	
DEBAINS & JUPILLE	90.3				81.3	
CORPER	23	14	37.4	7.2	27.6	20



## II

THE PRESENT INVESTIGATION.TECHNIQUE.

This in its essentials was based on the complement fixation technique already employed by Dr. Rutherford for the Wassermann Test in the Dispensary Laboratory.

SERUM. This is withdrawn from the tube after standing in contact with the clot for at least 24 hours. This increases the strength of the reaction, and according to Von Wedel<sup>53</sup> and Punch "tuberculous sera which have been kept for some time in this manner become positive though negative at the time of withdrawal, this not being true in the case of healthy sera.

COMPLEMENT. This is pooled guinea pig serum withdrawn on the evening previous to the test. This was found to be important as the titre is apt to change during the first twelve hours, and when the performance of the test continues for a whole day misleading results may occur if the complement is fresh. A dilution of 1/10 was used.

ANTIGEN. This was prepared according to Dr. A.C. Inman's instructions and is a modification of Besredka's second one, the advantage claimed being



a lessening of the liability to non-specific fixation. To prepare the antigen a mixed strain of tubercle bacilli is cultivated on Dorset's egg medium, being subsequently transferred to fresh tubes from time to time. Subcultures are made on glycerine potato, the glycerine solution containing .01% of gentian violet. After one month the culture is ready to be used as an antigen. It is transferred to a Haydn's mortar, ground with normal saline solution and made up into a saline suspension having an opacity equivalent to one containing 2,000,000,000 bacteria per c.c.

After comparing the antigenic values of living and dead bacilli the choice fell on the latter, both proving equally effective and the former being responsible for unpleasant reactions in those who prepared the emulsion.

Table 3 indicates the close correspondence between the two. Each antigen was of course titrated separately, and the data in the table were obtained over a period of three weeks.

Table 3/



Table 3.

		Living.	Dead
<u>Sputum positive</u> to <u>tubercle bacilli</u> <u>6 cases</u>	1	+ + +	+ + +
	2	+ + +	+ + +
	3	+ + +	+ + +
	4	Insuff. serum	+ + +
	5	: :	+ + +
	6	+ + +	+ + +
<u>Sputum negative</u> to <u>tubercle bacilli</u> <u>10 cases.</u>	1	-	-
	2	-	-
	3	-	-
	4	-	+ - -
	5	-	-
	6	-	-
	7	+ + +	+ + +
	8	-	-
	9	+ + -	+ + -
	10	+ - -	+ - (weaker)
<u>No Sputum</u> <u>2 Cases.</u>	1	+ + +	+ + +
	2	+ + +	+ + +
<u>Horse Serum</u> <u>Injections.</u> <u>2 Cases.</u> <u>v.p.</u>	1	+ Weak	+ Weak
	2	+ Weak	+ Weak
	1 <sup>st</sup> week	+ + - Weak	+ + - Strong
	2	+ - -	+ + -
	2 <sup>nd</sup> week	-	-
	1	-	-
	2	-	-
	3 <sup>rd</sup> week	-	-

HAEMOLYTIC SYSTEM. Rabbit v. Sheep serum

(Burroughs Wellcome & Co.) was used, this usually having a titre of 1/1000; five minimum haemolytic doses were employed. The haemolytic antigen was a 1% suspension of washed sheep corpuscles.



The Wassermann Reaction was invariably carried out in conjunction with the tuberculosis test.

Titration of Complement to ascertain its Minimum Haemolytic Dose was carried out each day. Titration of the freshly prepared Antigen with reference to its complement absorbing power was also necessary on every day on which tests were carried out. The suspension opacity was judged as correctly as possible, a sealed standard being employed to aid in this. Observations were always made using a constant quantity of the antigenic emulsion, say 0.3 to 0.5 c.c. with varying doses of Complement and also with three doses of Complement and varying amounts, e.g. 0.25 c.c. to 1 c.c. of antigen. A suitable dose of antigen having been thus ascertained, the actual test was put up in four tubes as indicated in Table 4 following.

Table 4.

	<u>Tube 1</u>	<u>Tube 2</u>	<u>Tube 3</u>	<u>Tube 4</u>
<u>SERUM</u>	0.1 cc.	0.1 cc.	0.1 cc	0.1cc
<u>COMPLEMENT</u>	$1\frac{1}{2}$ (M.H.D.)	2 (M.H.D.)	$2\frac{1}{2}$ (M.H.D)	3 (M.H.D)
<u>ANTIGEN</u>	Nil.	According to Titre		
<u>SALINE.</u>	Add 1 c.c.			



Incubate 1 hour 15 minutes; add to each tube 1 cc of diluted antishoop serum and 1 cc 1% blood corpuscles. Incubate for a further 60 minutes, shaking after 30. Read at the end of this period.

The antigen used is as a rule anticomplementary to the extent of 1 Minimum Haemolytic Dose; therefore if the serum to be tested can fix between 1 and  $1\frac{1}{2}$  Minimum Haemolytic Doses of complement itself the control will show complete haemolysis while tube 2 will give a weak positive reaction. Thus in the absence of a titration of the serum for anticomplementary properties such a weak positive is of little account.

The question of adding another  $\frac{1}{2}$  Minimum Haemolytic Dose of Complement to the other tubes was considered, but as a tuberculous serum has by no means the same fixing properties as a syphilitic one it was thought that the resulting maximum of  $3\frac{1}{2}$  Minimum Haemolytic Doses for tube 4 would be excessive. It was therefore decided to carry on according to the scheme set out above.



## III

PERSONAL REACTIONS DURING PREPARATION OF ANTIGEN.

During the course of the investigation a phenomenon was noted which has a direct bearing on the subject under discussion, and which is therefore set forth now in detail.

The antigen was prepared according to Inman's directions in the manner described above. The tubercle bacilli were transferred to a Haydn's mortar, ground with saline for about fifteen minutes previous to being transferred to the diluting fluid, and the various pieces of apparatus used were placed in a boiling water bath for safety: any tubes containing antigen which were subsequently discarded were treated in the same manner.

On the first occasion on which the test was carried out nothing exceptional was noted.

On the following Friday evening, however, the tests having been performed during the same day, certain symptoms were observed in "X" which were not at the time attributed to the work in hand. These were chilliness and shivering and pains in the knee joints, followed an hour later by nausea and dizziness and a temperature of 100.2°. This had subsided by the morning, though joint pains and lassitude continued till midday on the Sunday.



"Y" meanwhile had a similar experience, the temperature in this case attaining a maximum of 102° and the symptoms being correspondingly more pronounced.

Suspecting the work to be responsible, an identical technique was carried out on the following laboratory day with similar though more pronounced results, temperatures being respectively 101° and 103°.

On the third occasion more precautions were taken to avoid the possibility of inhalation or ingestion of bacilli, masks being worn and the actual grinding being performed under several thicknesses of gauze. A reaction still more severe supervened, however, temperatures being 102° and 104°, the symptoms beginning earlier and being characterised by much dizziness and nausea, and later sweating. A chart showing "Y's" reactions is exhibited below. The increased severity of symptoms in "Y" was accounted for by the fact that "Y" had been exposed for a longer period to the toxic influence.



Notes of Case.

Name {

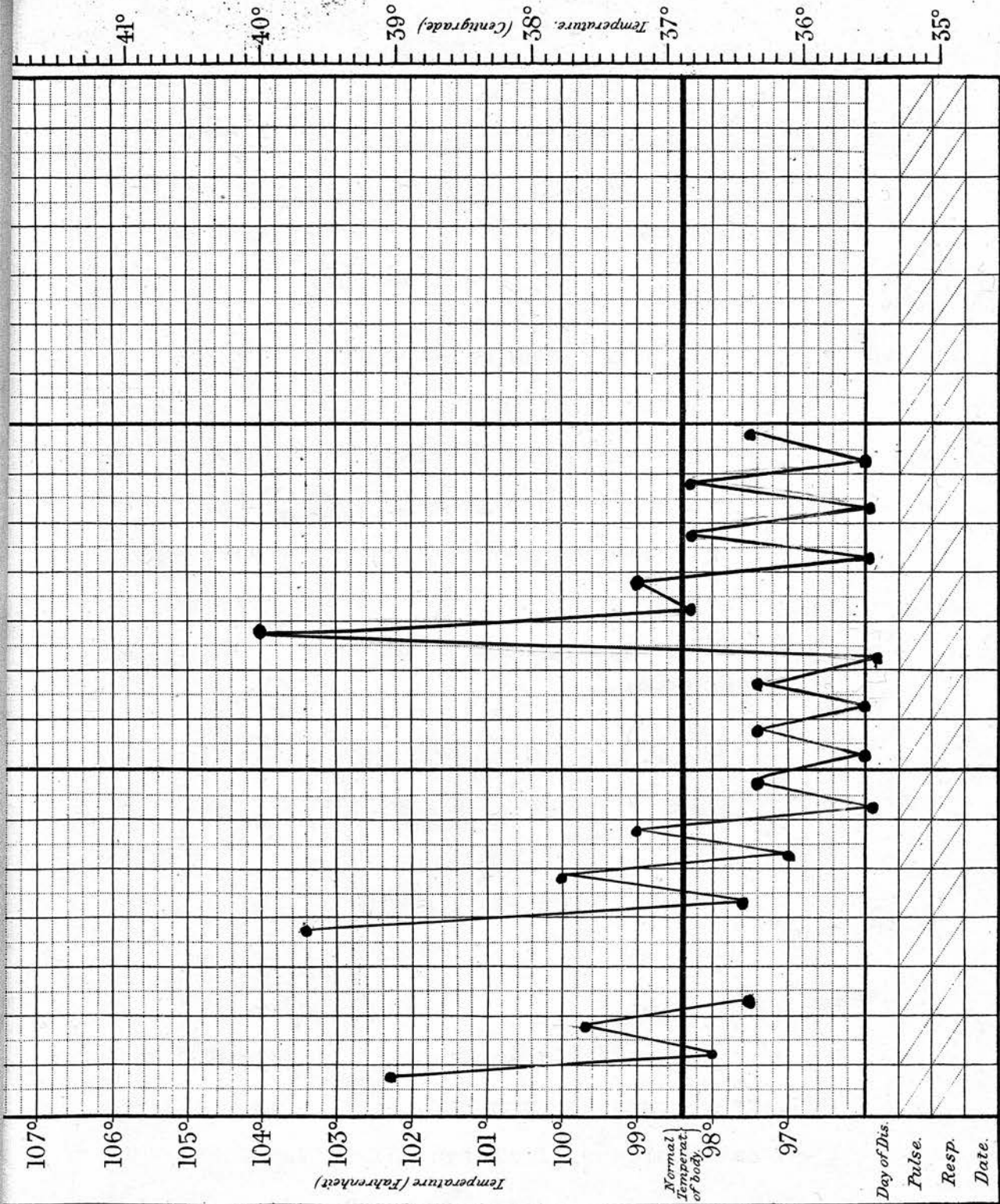
Age

Diet

Case Book No.

Date of admission.

Result





A special investigation to discover the cause was of immediate practical importance if the work was to continue along the lines determined, and was proceeded with as follows:-

(1) The culture to be used was killed on the day previous to the test, the grinding was performed on the sill of an open passage window and the instruments were placed in lysol instead of being boiled. No reaction whatever resulted.

(2) On the next occasion grinding was carried out in the laboratory. Otherwise the previous week's precautions were taken. No reaction followed.

(3) Next, living culture was used, ground in the laboratory, but other precautions remained as before. Still no result ensued, showing that the actual grinding had not been to blame.

(4) On the following occasion dead bacilli were used and boiled as at first, all windows being kept closed during the process. A slight response was noted on the part of "Y"; none on that of "X".

(5) Finally, the original technique was reverted to, all windows being securely closed as before "Y" showed a temperature of  $100.5^{\circ}$  during the following night.

On repetition the next week, nothing abnormal was noted on the part of either "X" or "Y".

Reference/



Reference may be made to Calmette<sup>54</sup> who states that certain pathologists are subject to such reactions and that they never become immune to them, but from the experience described above this would not appear to be the case. The cause he suggests to be a volatile toxin.

To summarise - (i) The reaction is produced by a volatile toxin contained in the living bacillus, probably not set free to any large extent by grinding, but driven off by heat. This would explain the slighter effect of the (already heated) dead culture (4 above).

(ii) Immunity to such reactions can, for a time at least, be established.



## IV.

THE DATA.

Sera from 508 cases were examined, including pulmonary and other forms of tuberculosis, and patients suffering from other diseases such as syphilis, gonorrhoea and malaria. Patients having other non-tubercular chest diseases were included, and a large number of presumably healthy bloods.

Complete clinical data were collected at the same time and records kept of these, so that a statistical correlation of the reaction to the various signs and symptoms might be attempted. (Table 5 gives general results in pulmonary tuberculosis.)

Table 5.PULMONARY TUBERCULOSIS.

241 Cases (clinically or bacteriologically diagnosed)

Condition of Lesion	Active	Quiescent	Doubtful
Number of Cases	113	100	28
Percentage of Positive Reactions	90.3	39	71.4
Amount of Lesion	Slight Involve-ment	Moderate Involve-ment.	Much Involve-ment.
Percentage of Positive Reactions	54.4 <i>active 89.7</i> <i>inactive 34</i>	76.5 <i>active 92.3</i> <i>inactive 43.5</i>	88.9



These include all cases diagnosed as pulmonary tuberculosis, on whatever grounds. Craig's<sup>26</sup> results under the same headings are consistently higher, particularly as regards "quiescent" cases. Miller's<sup>25</sup> also may be noted, namely 275 positive results out of 284 active cases, or 96.8%. These authors, however, are using their own special antigens and achieved readings which no one else obtained with them. Thus Moon<sup>27</sup> shows from 87.5% to 84.3% positive results with Miller's antigen, while Sellars and Ramsbottom<sup>39</sup> could make very little of Craig's.

On the whole, therefore, these results are as satisfactory and consistent as those of most writers, and may be accepted as suitable ones on which to base an investigation.

Table 6 shows the results according to the bacteriological findings in the sputum. Tables 7, 8, 9, record results relative to systemic disturbance, duration of disease, and occurrence of haemoptysis respectively. Throughout + means deviation of Complement in tube 2 in the actual test (v Table 4) and not in tubes 3 and 4, ++ deviation in tubes 2 and 3 only, and +++ indicates deviation in tubes 2, 3 and 4.

Table 6/



Table 6. - Results according to Sputum.

	<u>All cases</u>	<u>Active</u>	<u>Inactive</u>
Sputum positive to tubercle bacilli	90.8% +ve	95.8% +ve	62.5% +ve
Sputum Negative	45.7% +ve	69.6% +ve	34.5% +ve

Brown and Petroff,<sup>55</sup> using Petroff's glycerine extract antigen find 95% in active sputum - positive cases as against the above 95.8%, and 24% positive in non-active sputum negative patients as against the above 34.5%. In "all cases" their 90% corresponds closely with the above 90.8%.

Table 7. Results relative to Systemic Disturbance.

<u>Amount of Systemic Disturbance.</u>	<u>Number of Cases</u>	<u>Percentage Positive Reactions</u>
Slight	71	91.7%    ) 16.9%    ) 21.1%    ) 57.7%
Moderate	73	17.8%    ) 10.0%    ) 39.7%    ) 68.5%
Great	42	7.1%    ) 9.5%    ) 69.1%    ) 85.7%



Table 8. Results according to Length of Disease.

	less than 1 yr.	less than 5 yrs.	Over 5 yrs.
+	28.6%	14.4%	22.2%
++	9.5%	12.2%	11.1%
+++	33.3%	39.0%	48.2%
-	28.6%	34.4%	18.5%

or simplified -	+	-
1 yr.	71.4%	28.6%
5 yrs.	65.6%	34.4%
over 5 yrs.	81.5%	18.5%

Therefore the length of disease has apparently no influence on the reaction, though it is noticeable that a very strong one becomes more frequent as the age of the disease increases.

Table 9. Showing Results relative to Haemoptysis and Staining.

	Haemorrhage	Staining	Neither
+	18.2%	17.1%	15.1%
++	18.2%	8.6%	14.8%
+++	51.5%	42.9%	27.8%
-	22.1%	31.4%	42.8%

or

	Positive.	Negative.
Haemorrhage	87.9%	22.1%
Staining	68.6%	31.4%
Neither	57.2%	42.8%



Thus history of haemorrhage and degree of haemorrhage has a distinct relation to strength and frequency of reaction, and it is interesting to note that if it can be established below that this test has a prognostic value, the incidence of haemoptysis and staining in the course of the disease must tend to make the prognosis more grave. This is contrary to the opinion held by some, Fishberg<sup>56</sup> saying that if not immediately fatal it has no effect on the patient or the disease, and means nothing.

Relation of Strength of Reaction to  
Severity of Disease.

This is a vexed and rather important question, for some workers regularly use varying amounts of complement with each serum, while others regard such a procedure as useless. W.O. Meek<sup>57</sup> states that on the whole the more advanced cases give stronger reactions, but that this cannot be relied on: also that there is no relation to exacerbations, and no relation to sustained improvement. Dudgeon, Meek and Weir<sup>23</sup> say that there is no practical value in the procedure, though weakening often coincides with clinical improvement. Craig<sup>37</sup> used varying quantities, and Punch,<sup>11</sup> while Inman<sup>4</sup> achieved his quantitative readings by varying dilutions of serum only, the complement remaining constant.



Taking into consideration these differences of opinion, the subject merits examination at some length.

For the sake of simplicity in the following table, (Table 10) degrees of involvement are classed as I, II, and III, and systemic disturbance is divided into three categories, a, b, and c.

Table 10. - Degree of Involvement.

	I.	II.	III
	<u>29 cases</u>		
1. +	66%	31%	3%
	<u>32 cases</u>		
++	31%	62%	7%
	<u>76 cases</u>		
+++	27%	51%	22%

Systemic Disturbance.

	a.	b.	c.
	<u>28 cases.</u>		
2. +	54%	39%	7%
++	41%	38%	21%
+++	21%	37%	42%

Progress of disease.

	IMPROVING	STEADY.	LOSING
3. +	54%	42%	4%
++	59%	29%	12%
+++	22%	20%	58%



Analysing this we see that under (1) the + series is largely composed of slightly involved cases, and the +++ contains far more extensive cases than either of the others. Under (2) exactly the same applies to severity of general symptoms. Under (3) the majority of + and ++ cases are improving or stationary, while most of the +++ cases are going down.

After considering the facts set forth above, it seems to be established that the use of varying quantities of complement is of real value.

Surgical Tuberculosis. The results are shown in Table 11 in active and quiescent cases and according to duration of disease.

Table 11.

	+	++	+++	Total %age +ve	-ve.
Active 26 cases	23.1%	15.4%	26.9%	65.4%	34.6%
Quiescent 9 cases	0	0	0	0	100%

According to Duration of Disease.

	1 yr.	5 yrs.	Over 5 yrs.
+ve.	40%	54%	50%
-ve	60%	46%	50%



These results approximate to those of Dudgeon, Meek and Weir<sup>23</sup> and give a lower percentage than McIntosh's,<sup>10</sup> Fried's,<sup>20</sup> and some others. Mozer and Fried<sup>27</sup> obtain a very much higher percentage in earlier cases than in later ones, but that has not been the experience in the present series, as shown by the table above.

### Results in Glandular Tuberculosis.

Seven cases were tested: all were positive four giving results. These were all undoubted cases of glandular tuberculosis, and though few in number the findings were very constant. Other published results vary from 37.5% (McIntosh),<sup>10</sup> to 70.7% (Mozer and Fried),<sup>27</sup> and 71.4% (Courcoux)<sup>7</sup>

### Skin Tuberculosis.

Four sera were tested and all were negative. In two cases there was much destruction, in the others only moderate involvement. These were consistently negative, though re-examined. Ichok, Goldenberg and Fried<sup>6</sup> found 66.4% definitely positive in such cases, though they state that "Capelli denies that this is so."

Other Diseases. Of the sera from eleven cases visiting the Dispensary, and already diagnosed as bronchitis, aortitis, asthma, unresolved pneumonia, bronchiectasis and post influenzal debility, all were negative.



One diagnosed as "mitral disease" and having no clinical signs of tuberculosis on physical examination but showing a right apical haziness on the screen, gave + - -.

Healthy Individuals. Seventy sera collected from people known to be healthy, or from those, where examination was impossible, whose health there was no reason to doubt: all gave negative results.



## V.

NON SPECIFIC REACTIONS.A. SYPHILIS.

The question of a false positive reaction resulting from the serum of a non tuberculous syphilitic patient is the most important to be decided under this heading, owing to the wide distribution of the latter disease. Being taken for the most part from a tuberculosis dispensary practice, these results require full analysis. (Note "W.R." means Wassermann Reaction: and "C.F.T." means Complement Fixation Test for Tuberculosis throughout).

There were 41 cases involved: 20 of those were positive to both tests: 21 were Wassermann positive and negative to C.F.T. They are considered hereunder in four groups.

Group I. Of many sera collected from Ward 5A of the Royal Infirmary, from patients undergoing specific treatment, four gave a positive W.R. and a negative C.F.T. (4 Cases: W.R. +ve, C.F.T. -ve).

Group II. Of dispensary patients diagnosed as having diseases other than tuberculosis, or as "clear" there were 8 cases which were Wassermann positive and negative to the other test. ( 8 cases: W.R. +ve, C.F.T. -ve).

Group III/



Group III consists of Dispensary Patients, having Quiescent pulmonary tuberculosis. There were 15 cases of which 7 gave W.R.+ve, and C.F.T. -ve: the remainder were positive to both tests.

Group IV, is made up of Dispensary Patients, having Active pulmonary tuberculosis. Of 14 cases 2 were W.R.+ve, C.F.T. -ve, and 12 were W.R.+ve C.F.T.+ve.

Taking groups III and IV, and comparing them with the general data given before, we find as in Table

Table 12

%age +ve to C.F.T.

Pulmonary Tuberculosis Quiescent.	W.R.+ve 15 cases	53.3% +
	General Series 100 cases	39.0% +
Pulmonary Tuberculosis Active.	W.R.+ve 14 cases	85.7% +
	General Series 113 cases	90.3% +

Considering the limited numbers of Wassermann positive sera there is very close correspondence here, and therefore reviewing each of the four above classes there can be no doubt that the co-existence of syphilis does not influence the tuberculosis reaction when Inman's antigen is used.

It/



It is hardly necessary to state that the diagnosis of the various cases mentioned above was made independently of the test, and previous to it.

These results correspond with the claim made by Inman, by Punch and Gosse<sup>44</sup> using a similar antigen and by Miller,<sup>25</sup> and agree with the conclusions reached by Watkins and Boynton.<sup>36</sup> They appear to be more satisfactory than those obtained by use of the extract antigens (Inman,<sup>42</sup> McCaskey,<sup>32</sup> Besredka and Manoukhine,<sup>38</sup> and others).

There remains the question of the false positive Wassermann stated by some (Snow and Cooper,<sup>73</sup> Moursound<sup>35</sup>) to be obtained in tuberculous non syphilitic patients.

It was the routine practice to send these patients to Ward 5A. R.I.E. whether tuberculous or not, and every one who went there was accepted for treatment after examination. Of those whom it was practicable to interrogate one only denied a syphilitic history, and therefore the absence of this non specific action may be assumed.

#### B. MALARIA.

Malaria has long been regarded as a source of non specific positive results in the Wassermann Reaction and the same is said to be true of the Tuberculosis Complement Fixation Test. Thus Rieul and/



and Bass<sup>16</sup> found that readings were confusing and unreliable in tuberculosis in presence of either of these diseases. Dr. David Lees has investigated this subject with regard to the connection between malaria and syphilis, and informs me that in the absence of an acute exacerbation of the former disease, no cross fixation exists. Dr. Andrew Rutherford tells me that his experience of about 3000 Wassermann Tests in India is in agreement with this.

It was therefore decided to inquire into the relationship between malaria and tuberculosis on the same lines. Unfortunately only five sera were available from patients who were in hospital for malaria, but who were not suffering from an acute attack at the time of taking the specimen. These were all negative to both W.R. and C.F.T.

It may therefore be concluded that the same is true of tuberculosis as of syphilis: that Malaria in its apyrexial stages has no influence on the test, and that during pyrexia it is usually easily recognised and therefore of minor importance in this connection.

C. DIPHTHERIA/



C. DIPHTHERIA.

That patients who have recently suffered from diphtheria often give a positive fixation in the absence of tuberculosis has been shown by several. Urbain and Fried found definitely positive, though not very strong, results under these circumstances, and Cooke found the reaction unreliable in convalescent diphtheritic subjects. On the other hand Urbain and Fried<sup>59</sup> made an antigen of diphtheria bacilli and obtained positive readings with tuberculous serum, and those of Negre and Bouquet<sup>63</sup> with diphtheritic and tuberculous antigen were indistinguishable in strength. The former observation is of importance and falls to be discussed here.

Cooke attributes his positives to the fact that antidiphtheritic serum had been given: Urbain and Fried make the same observation, accounting for the result by the administration of antitoxin three to twelve days previously. The results obtained in the present investigation are shown in Table 13 below.

Table 13

	+	-
Antitoxin 10-20 Days ago	7	2
20-30 Days ago	2	0

	+	-
Antitoxin. Less than 3000 units	7	1
More than 3000 units	2	1



Again the sera tested are not numerous, but there are enough on which to base a conclusion. It would have been a great advantage to have had sera from diphtheria patients taken before administration of antitoxin, but this for obvious reasons was not possible.

The length of time elapsing between the administration of the antitoxin and the taking of the blood has apparently no effect on the reaction after ten days, and the number of units given is immaterial.

The question now arises:- on what grounds do the authors quoted above put the responsibility for the positive result on the antitoxin? Cooke finds his reading confused in treated diphtheria patients, which means that the sera from these may give a non-specific reaction as strong as may be expected in a tuberculous patient: Urbain and Fried find that diphtheritic blood (untreated) gives a positive, but only one tenth as strong as a tuberculous serum. At first glance, therefore, it would seem that the administration of antitoxin has considerably enhanced the strength of the reaction.

Now/



Now the blood is non-specifically (weakly) positive in diphtheria by virtue of the antibodies produced by the disease: but it is inconceivable that ready made amboceptor added from an external source can have anything but a fleeting existence in the blood stream, or be capable of influencing the reaction for more than a few hours. It is therefore difficult to understand why the above authors should attribute positive findings, following for weeks afterwards, to its agency. It was thought that the horse serum, apart altogether from the antitoxin, might offer a solution to the problem, and injections of 10 cc were given to two individuals who had consistently negative sera and who happened to be available for the purpose. Dispensary patients to any number could not of course be pressed into volunteering for this service. Results are shown in the accompanying table.

Time after injection.

	40 hrs.	9 Days	16 Days	23 Days	30 Days
X	-	+	+++	++	-
Y	-	+	No Test	++	-

The/



The above is striking proof that normal horse serum has an effect of its own, and suggests that antitoxin per se has nothing to do with the reaction: these two bloods were used as controls frequently during the eighteen months of the investigation and were only positive on one other occasion nine months later, when made so artificially by injections of tuberculin.

In conclusion two antidiphtheritic sera, undiluted and diluted 1 in 10, were tested, each taken from tubes containing 2,000 units. Results were as shown in table below.

	Undiluted	Dil 1/10
a	++	-
b	+	-

Thus a moderately strong positive is found in the undiluted serum, but a 1/10 dilution is enough to change this to a negative. Injected into the blood stream, there is a process of simple dilution of diphtheritic antibodies (and to a far greater extent than in the above experimental tests). It is therefore difficult to see how these highly diluted antibodies can affect the reaction.

It/



It may then be taken that Diphtheria causes a serum to give a weak positive reaction in the tuberculosis test. Injection of antitoxin changes this to a stronger one, but the change is due to the serum, and not to the antitoxin contained therein.



INFLUENCE OF TUBERCULIN INJECTIONSON THE TEST.

On this question, as in most aspects of the subject, conflicting statements are found in the literature. The earlier workers found that such treatment made the serum positive (Wassermann and Bruck<sup>1</sup>) but as their antigens were tuberculins, this observation is of little interest here. Dudgeon, Meek and Weir<sup>23</sup> found that all tuberculin treated patients were positive, and that the strength of reaction appeared to vary directly with the dosage. Later Dudgeon,<sup>24</sup> experimenting with rabbits, found that after a course of bacillary emulsion in infected animals the strength of reaction was not increased, but reduced. Dudgeon, Meek and Weir<sup>25</sup> state that there is no constant or marked effect on the amount of immune body, but that in some cases administration has been associated with improvement and loss of antibodies from the blood. Calmette and Massol<sup>36</sup> using different tuberculins found one, a watery extract, whose administration produces much immune body, and they state that therefore the antibodies in the sera of tuberculin treated patients are of real interest.

During/



During the period of this investigation as many suitable cases as possible were put on tuberculin (Bacillary Emulsion) with a view to discovering the truth of the matter.

The "survivors" of a tuberculin course in Dispensary practice always constitute but a small percentage of those who set out on the treatment. Some leave town, some, alarmed at a sore arm, fail to report, and others lose interest and cease to attend. The latter after tactful home visitation may appear spasmodically for a few weeks and then lapse, so it will be understood that the eighteen cases reported represent a lesser number than originally was hoped for. Results obtained are noted in Table 14 below.



TABLE 14

Note: Original solution of Bacillary emulsion contains 5 mg per c.c.: Dil. I contains .5 mg in 1 c.c. and so on.

Patient	Test before course	Final Injection	Test at end
1	-	Dil II .2 cc	-
2	-	Dil III .5 cc	-
3	+++	Dil III .5 cc	+++
4	+++	Dil III .8 cc	+++
5	++-	Dil II .2 cc	++-
6	-	Dil II .1 cc	-
7	-	Dil I .8 cc	+++
8	-	Dil I .1 cc	+++
9	+++	Dil II .5 cc	++-
10	+++	Dil I .9 cc	+++
11	-	Dil III .7 cc	-
12	-	Dil I .5 cc	++-
13	-	Dil II .5 cc	+-
14	+++	Dil II .5 cc	+++
15	-	Dil II .1 cc	-
16	+-	Dil II .1 cc	-
17	+++	.1cc orig. sol.	+++
18	-	Dil I .5 cc	+-
			2 months after cessation.

More shortly

a	Constantly negative throughout	5
b	Constantly positive throughout	5
c	Stronger at end	6
d	Weaker at end	2



The changes indicated in (a) are so slight as to be accounted for by the normal variations in the test, but even though many of the courses are incomplete this could hardly be said of (c) and it must be concluded that the injections have increased the antibodies in the blood.

To confirm this further a course was taken by two individuals (both healthy) using a quicker increase of dosage than would be permissible in a dispensary patient, the last dose in the case of "X" being followed by a temperature of 100, and in that of "Y" by 102. Results after varying intervals following the last injection are shown in Table 15

TABLE 15

	Before	After last injec.	7 Dys.	14 Dys.	21 Dys.	28 Dys.	35 Dys.	42 Dys.
"X"	-	"X"	++ -	+++	+++		-	-
"Y"	-	"Y"	++ -	++ -	-	-		

It would seem therefore that tuberculin gives the advantage of an immunity in the blood such as is produced by the disease but without any of its attendant disadvantages, and the question of prophylactic treatment naturally arises. Some have attempted to reproduce the natural immunity of certain animals, notably the gerbil, by non-specific/



specific methods. Barbary<sup>41</sup> used cinnamates and Rappin<sup>42</sup> a vaccine containing tubercle bacilli treated with antituberculous serum, made up with a solution of sodium fluoride. Others have tried sodium gynocardate and sodium morrhuate, but we are more concerned with the specific methods. Nathan Raw<sup>43,44,45</sup> reports excellent results with his vaccine prepared from attenuated cultures, and Sir R.W. Philip<sup>46,47</sup> finds a remarkable improvement, both local and general, in cases of glandular tuberculosis treated by inunction. Shiga<sup>48</sup> also urges protective treatment early in life, and quoted his experience to substantiate his views.

Reviewing this literature the case for prophylactic treatment is strong, and whether the antibodies demonstrated above represent actual immunity or merely an indication of its presence (Martinette<sup>49</sup> is immaterial so long as the immunity is there. The length of time that this will be effective can be taken to be considerably longer than the twenty-one days of positive reaction shown in Table 15 for the average healthy adult possesses immunity though having no demonstrable antibodies. Surely then we cannot be too insistent on the need for protective treatment of children of tuberculous families and infants of actively tuberculous mothers, whose chances of life at present are very often greatly diminished.



## VII.

"REACTION OF ANTIGEN".

Though time was not available for work along the lines of Debré and Paraf<sup>71</sup> and Anché and Portman,<sup>72</sup> their investigations have a direct bearing on the subject matter of this thesis, and require brief discussion here. Recognising that in tuberculous disease antigen may at times already be in excess in the fluid to be examined and that antibody may be absent, they performed the test supplying the latter instead of the former. Various fluids were used, those from pleuritic and ascitic cases, and urines, by Debré and Paraf<sup>70</sup>; and milk by Anché and Portman.<sup>72</sup>

In this technique the antibody is provided by the serum of an actively tuberculous patient, in which its presence has previously been ascertained. Debré and Paraf report strikingly good results with pleuritic exudates, in thirty-two cases every finding being confirmed by the subsequent course of events. This is what one might be led to expect, for the effusion is at the site of the active lesion and therefore is a source from which concentrated antigen is being introduced into the blood stream; if the organism is successfully dealing with the/



the infection the antibody will be in ~~ex~~cess in the general circulation, but not at the local centre of activity. Courcoux<sup>17</sup> states that sometimes tuberculous fluids examined were negative and the bloods positive, though Ogawa<sup>4</sup> finds a large percentage positive. In my own experience pleural effusions have been negative in undoubtedly tuberculous subjects, though the numbers are not large enough for reference.

Of three cerebro-spinal fluids from patients dying of tuberculous meningitis in the Sick Children's Hospital two were negative and one weakly positive, and the negative reaction in the blood of some advanced tuberculous patients is a well known phenomenon. The explanation usually adduced is the same, namely already existing excess of antigen.

It would appear therefore that if Debré and Paraf's modification were used in conjunction with the previously described test, joint effusions, exudates, cerebro-spinal fluids and suspected fluids of all kinds might afford very valuable data in a field which at present is generally neglected.



## VIII.

VALUE OF THE COMPLEMENT FIXATION TEST IN DIAGNOSIS.

The results quoted earlier in this paper have already shown the test to have some diagnostic value, but the analysis of a few picked cases detailed below should be of interest in showing its practical application.

1. P.M. Boy, aet 7, examined at Dispensary first four years ago, the following notes being recorded:-

Complains of breathlessness, cough, weakness, and failure to thrive. Has been gradually getting worse for last two years.

Examination:- Impairment of note at left apex and left axillary region, fine rales here and anteriorly to this. Posteriorly rales in L interscapular region spreading towards base and a few in R. interscapular space. Diagnosis, Pulmonary (Hilus?) Tuberculosis. When seen four years later, having reported for examination, the serological test was negative and the report reads "Moisture persists at bases, L apex still impaired. Cough very troublesome with spit. General condition good and at school. Diagnosis: Bronchitis with bronchiectatic condition in both bases."



It certainly would have been more effective to have tested the blood at the time of the first diagnosis, and to observe thereafter the progress of the disease. Such a course was, however, impossible in an investigation limited to a year and a half, so the method followed in Case 1 was used in such cases. The child whose blood was negative four years later must in all probability have been negative at his original visit, for the tuberculosis simulated was advanced and his subsequent history shows that he never had it

2. W. McI., aet 56, male, was suspected to have a syphilitic gumma of the 5th right rib in the mid clavicular line. Examination of his blood showed, very unexpectedly, W.R. -, C.F.T.  $\text{+++}$ . At the operation what had originally been thought a gumma turned out to be a cold abscess originating from a tuberculous rib, part of which was resected. (Recovery was satisfactory. Three months after operation when healing was entire the C.F.T. gave a  $\text{+++}$  result again.

3. G.S., aet 55. Admitted 1/2/23 with diagnosis of pulmonary tuberculosis from his medical attendant.

Complains of cough and spit and pain in left side of chest between second and fourth intercostal spaces. Sputum streaky, losing weight. Pulse/



Pulse 104. No tubercle bacilli in sputum.

Physical examination:-

Nothing notable on inspection. Percussion shows marked impairment over left apex and left parasternal region. Immediate diagnosis - "Pulmonary Tuberculosis. Serologically W.R.+++ C.F.T.-. X ray shows dense shadow in left side of chest, with rounded and more or less sharply defined lower border, extending to lower margin of second rib.

Though definite classical signs and symptoms of Thoracic Aneurism were absent, this condition was diagnosed on the strength of a full general examination and of the X ray appearances.

4. J.S., first seen in May 1918 when 7 years of age. Complains of cough and spit and enlarged glands in neck. History of pneumonia and whooping cough at 14 months of age, and pleurisy and pneumonia two years ago. Weight 39 lbs.

Examination shows impairment at both apices, marked Harrison's sulcus, moist sounds R. upper lobe both anteriorly and posteriorly, and both bases posteriorly. Diagnosis: Pulmonary Tuberculosis.

Seen in Feb. 1923, age 11. The complement fixation test was entirely negative.

Physical/.



Physical signs:- Moist rales persist under right clavicle and second right interspace, and slight percussion dullness noted in right upper lobe. Pulse 72, Wt. 59 lbs, at school and general condition very satisfactory. Now diagnosed as a chronic bronchitic condition.

5. J.S., aet 46. first seen 14/3/23.  
Has not felt well for past three months, but is still at work. Has a history of occasional staining, and complains of cough, spit and breathlessness. Sputum negative. Physical signs show no flattening, diminution of expansion or impairment, and no alteration of breath sounds except faintness in both lower lobes posteriorly. C.F.T. reads +++. X ray shows marked shadow springing from right root and spreading outwards and downwards towards chest wall, though not reaching to the periphery. Diagnosis, chiefly on strength of screen findings, Pulmonary Tuberculosis.

6. E.G. First attended Dispensary in May 1919, at the age of  $8\frac{1}{2}$ , weight 56 lbs. Complains of cough and spit and general weakness, looks pale and ill. Had pneumonia 3 years ago. Pulse 120. Moist rales front of both lungs and axillary regions, extending towards bases. Diagnosis/.



Diagnosis - "Observation". Seen in Nov. 1922. Age 11, weight 78 lbs. Cough and spit troublesome. Moisture throughout R. lung and at both bases. Diagnosis now Chronic Bronchitis.

7.J.L. Reported at Dispensary about Dec. 1920. History of occasional haemoptysis since 1915. Pulse 100. Tubercle bacilli absent from sputum. Has been unemployed for one year but says he feels fit to work. Physical examination reveals only slight percussion impairment in L. apex. Examination of blood showed C.F.T.+++.

In March 1923, his condition was as follows:-

Weight had fallen 11 lbs: cough increased with sticky sputum: impairment extended and moisture throughout upper half of both lungs. Sputum positive to tubercle bacilli.

8. J.M., aet 25. Notified by practitioner for removal as suffering from tuberculosis of lungs and spine. The Complement Fixation Test was entirely negative.

On examination this patient was found to have combined scoliosis and kyphosis of the dorsal vertebrae. The right apical percussion note was impaired but on account of the deformity no importance could be attached to this. Moist rales were present in right upper and both lower lobes. Temperature was normal and Pulse 86. Diagnosed as Simple Scoliosis and Bronchitis.



9. H.R. A female patient aged 26 had attended the Dispensary for some time for the treatment of Tuberculous Cervical Adenitis. Her glands had apparently subsided and become quiescent, yet even at the beginning of a course of tuberculin injections at the period under discussion, her serum gave a+++ reaction to the Complement Fixation Test. Some months later she complained of difficulty in walking and pain in the lumbar region, and on examination was found to have a spastic paresis of both lower limbs. There was no deformity or rigidity at the time, and the patient was admitted to the Royal Infirmary. On referring to her case sheets six months later (after discharge) the diagnosis is found to be "? Disseminated Sclerosis; Potts Curvature of Lumbar Spine".

When the patient was sent to the Infirmary it was considered that Disseminated Sclerosis had been excluded: unfortunately the result of the test was never sent to the authorities there, so it is impossible to quote their opinion considered in the light of this observation.

This case is put forward to demonstrate how the test can be of real value in directing the line of inquiry in certain difficult cases.



10. E.L. Age 12. First seen 3/1/21, complaining of cough with white sputum and a lump in her neck. Examination shows a fluctuating swelling on the left side of the neck and impairment of percussion note over the right lung anteriorly to the level of the 2nd space, and posteriorly at the apex. Medium rales right upper lobe front and back. Pulse 82. Diagnosis - Pulmonary Tuberculosis and Adenitis. March 1923; C.F.T. negative. Very healthy child, age 14, weight 126 lbs. Pulse 84. Slight cough: never tired: no complaints of any kind. Physical signs - Breath Sounds still bronchial at R. apex. Slight impairment R. upper lobe and moisture throughout right lung. Condition not Pulmonary Tuberculosis.

11. M.L., female aet 9. Has been losing weight for some time, is thin and poorly nourished, and is cyanosed. Physical examination shows cavitation in left apex with moisture, and dry crepitations in right upper lobe. Sputum contains tubercle bacilli. C.F.T.+++Report at time of discharge four months after admission "Not improved".

12. M.D. Girl aged 9, had been off school for two months complaining of lassitude, loss of appetite, and slight cough. Physical examination shows cavitation in Right apex, and sputum is positive/





positive to tubercle bacilli. C.F.T. +++

Discharged 4 months after admission, patient having gained 5 lbs., and recorded as "improved".

It would therefore appear that the test can be of considerable value to the clinician in forming his opinion. It seems especially useful in cases with more or less advanced disease and few clinical signs, as in Hilus Tuberculosis ( see Case 5 above). In children suffering from well marked disease whose nature gives rise to doubt, it is also of considerable help. Cases 11 and 12 are included, not because there was any difficulty in diagnosis, but to show that the blood may be expected to be positive in children if tuberculosis is present. It might be objected that the test in children is not reliable, and in the case of the very young this is true. Reference to this side of the question is very scanty in the literature.

Bauer (quoted by Cooke, *vide infra*) found all of sixty-one sera from children, some tuberculous and some healthy, to be negative. The antigen, however, was a tuberculin one.

Heiman<sup>44</sup>/



Heiman<sup>44</sup> publishes the following results,  
using Miller's antigen.

C.F.T.

50 Children Aet 6 months to 12 years.	17 Tuberculous	2 positive
	5 Probably Tuberculous	All negative
	28 Non Tuberculous	3 positive.

the significance of which appears difficult to  
evaluate. Cooke<sup>43</sup> investigated the question more  
thoroughly and found the following in a large series.

1st year	all negative
2 - 4 years	40% positive
4 - 6 years	56% do.
Over 6 years	80% do.

This is reasonable and corresponds with the  
degree of resistance to tuberculosis which we know  
to exist in later childhood.



## IX.

VALUE IN PROGNOSIS.

It is well in the course of such a discussion as this to consider again from time to time how much we may expect from our labours.

The tuberculosis test, admittedly less reliable than the Wassermann, is expected by some writers to give even more information. The Wassermann Test is not called on to furnish a guide to prognosis so much as for diagnosis, and with it positive means disease, and negative is very often taken to mean absence of disease. The test which forms the subject of this thesis, however, is claimed by some to furnish both diagnosis and prognosis; small wonder if it sometimes fails in one or the other. We cannot expect that it will indicate "positive" - meaning presence of disease, - and "negative" - meaning that recovery may be expected, at one and the same time. Any test which can give a completely reliable answer to one of those questions must of necessity be somewhat valueless in the other: one which attempts to answer both cannot be completely reliable in either. Bearing these facts in mind we may proceed to see what hints with regard to prognosis may be obtained.



The period available for research being too short for the employment of the obvious method of inquiring into this point, i.e.- re-examination after a period of years, an alternative plan was followed. This consisted in determining the general condition of each patient from pulse, temperature, clinical observations and history, and discovering what relation could be established between these findings and those of the test.

Of 82 patients in Hospital 54 were found to be gaining, 7 stationary, and 21 losing. Percentage positive and negative results to the C.F.T. are shown in Table 16

Table 16

	General Condition	Complement Negative	Fixation test Positive
<u>HOSPITAL</u>	Gaining (54)	46.3%	53.7%
<u>PATIENTS.</u>	Stationary (7)	28.6%	71.4%
	Losing (21)	23.5%	76.5%

Of the negatives, 80.6% were gaining  
 6.5% : stationary  
 12.9% : losing

and of the +++ cases

45.8% were gaining  
 8.4% : stationary  
 45.8% : losing.

of/



Of 75 Dispensary patients 28 cases were gaining, 31 stationary and 16 losing. See Table 17 below.

Table 17

	General Condition	Complement	Fixation test
		Negative.	Positive.
<u>DISPENSARY</u> <u>PATIENTS</u>	Gaining (28)	53.6%	46.4%
	Stationary (31)	32.3%	67.7%
	Losing (16)	6.3%	93.7%

Of the negatives, 57.7% were gaining  
38.5% : stationary  
3.8% : losing

and of the +++ cases

17.9% were gaining  
35.7% : stationary  
46.4% : losing

Reviewing these results we find that in both Hospital and Dispensary the "Gaining" cases contain the greatest proportion of negatives, and the "Losing" the largest number of positives. We also find that both tables show many positive "Gaining" cases but unfortunately this can not be taken to indicate a good prognosis, for a gain over such a short period as three or six months is of little importance in this connection. On the other hand six months is too often amply sufficient in which to determine the condition of a losing case, and the 76.5% and 93.7% of the preceding tables are clearly of significance.

There/



There are some interesting differences to be noted in the Hospital and Dispensary tables.

In the former (Table 16 ) the percentage of "positive gaining" cases is greater than in the latter, which is to be expected in view of the extra care and supervision which an institution affords. The "negative losing" figure in Table 16 however, exceeds that shown in the Table 17, because it includes some of the far advanced cases whose response to infection is too feeble for the production of antibodies, whereas in Dispensary Practice such patients do not attend the clinics.

Incidentally these tables afford a striking testimony to the efficiency of institutional treatment, for the Table 16 shows 65.9% "gaining" and 34.1% "holding or losing", while figures of Table 17 are respectively 37.3% and 62.7%.



## X.

SUMMARY AND CONCLUSIONS.

An investigation was carried out to determine the value of Complement Fixation in Tuberculosis. 500 Sera were tested, and Inman's antigen was used.

In the course of preparation of the antigen a general reaction was produced in the laboratory workers, which was probably due to a volatile toxin set free from the bacilli. Immunity can be established, temporarily at least, to this toxin.

The test gave satisfactory results in pulmonary, surgical and glandular tuberculosis. In tuberculosis of the skin it was always negative.

In patients suffering from other diseases, and in sera from healthy people, it was always negative.

Non-specific reactions do not occur with syphilitic sera when Inman's antigen is used. They do not occur in Malarial subjects who are temporarily free of acute attacks of this disease.

The non-specific positive reactions which are found in Diphtheria are due mostly to the horse serum/



serum injected, and not at all to the antitoxin of which it is the vehicle.

Tuberculin treatment produces antibodies in the blood which are demonstrable by the test, and this is regarded as practical evidence of the value of tuberculin in prophylaxis.

The "Reaction of the Antigen" would be a valuable accompaniment of the test in advanced cases, and some others.

The test has an important diagnostic and prognostic application. It can be of great use in many ways, as has been shown above, but it does not do more than take a place among the more significant "symptoms" of the disease, to be weighed in conjunction with, and in the light of, other observations.

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